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TWO SPECIES OF PEGOMYIA MINING THE LEAVES OF DOCK [1]

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INTRODUCTION

The life history and habits of two leaf-mining Anthomyids (*Pegomyia* spp.) are presented in this paper for the first time. The docks *Rumex crispus* L. and *R. obtusifolius* L. are extensively mined by several species of Pegomyia. Among these are two [3], *P. calyptrata* Zett. and *P. affinis* Stein., which occur commonly throughout the United States. *P. calyptrata* Zett., is by far the more common of the two species. The adult is readily distinguished by a bluish-gray thorax and a reddish yellow abdomen. *P. affinis* Stein., on the other hand, is less common. It occurs about Ithaca, N. Y., abundantly in early summer, but later in the season no eggs, larvæ, or adults have been found. This species is distinguished by its inconspicuous gray color.

THE MORE COMMON SPECIES, PEGOMYIA CALYPTRATA

HISTORICAL REVIEW

P. calyptrata Zett. was first described by Zetterstedt (1846) [4] since that time there have been but few references to it. Stein (1897),[5] (1907),[6] and Pandellé (1901)[7] refer to this species, but mention nothing regarding its habits. This species has undoubtedly been noticed by many, but its identity has been unknown. In looking over some unidentified material in the collection at Cornell University the writer found specimens reared by Prof. Comstock as early as 1882. Dr. A. O. Johannsen, also of Cornell, noticed the same species in 1913 mining dock, but did not publish his observations.

[1] Contribution from the Entomological Department of the Cornell University Agricultural Experiment Station.

[2] The writer wishes to acknowledge his indebtedness to Dr. Robert Matheson, of the Department of Entomology, Cornell University, for many helpful suggestions and the criticism of this paper.

[3] There are at least two other species of Pegomyia occurring in this country, *Pegomyia bicolor* Wied. and *Pegomyia winthemi* Meig., which mine *Rumex* spp. These occur in the northern United States and Canada, but do not appear to be common at Ithaca, N. Y.

[4] ZETTERSTEDT, J. W. DIPTERA SCANDINAVIÆ. V. 5, p. 159. Lundæ. 1846.

[5] STEIN, P. NORDAMERIKANISCHE ANTHOMYIDEN . . . In Berlin. Ent. Zeitschr., Bd. 42 (1897), Heft 3/4, p. 239-241, 286. 1898.

[6] BECKER, Th., BEZZI, M., KERTÉSZ, K., STEIN, P. KATALOG DER PALÄARKTISCHEN DIPTEREN . . . V. 3, p. 701. Budapest. 1907.

[7] PANDELLÉ, L. ETUDES SUR LES MUSCIDES DE FRANCE. In Rev. Ent. France, t. 20, no. 1, p. 294. 1901.

DISTRIBUTION

P. calyptrata Zett., occurs in Europe as well as America, although it appears to be exceedingly rare in the former. Stein (1906)[1] neglects to list this species in his paper. Zetterstedt (1846)[2] mentions its occurrence in Sweden, stating that one specimen was taken near Lund by Mr. D. Dahlbom and a second at Vadstena by himself. Two specimens were also taken at Altenburg, Germany. In America it is a common species, especially in New York State, where the author has found it widely distributed. Stein (1897)[3] mentions its occurence in Washington, Minnesota, Illinois, Pennsylvania, and Massachusetts. To these the writer adds New Jersey and New York, having taken the the species at Ithaca, Binghamton, and Tarrytown, N. Y., and Orange, N. J.

HOST PLANTS

P. calyptrata mines exclusively in the leaves of several species of Rumex. Adults have been reared from *R. obtusifolius* and *R. crispus*. Both species of Rumex are equally susceptible to the attack of the miner. *R. acetosa* is evidently a third host plant. Eggs of *P. calyptrata* were found on this plant. They hatched and the young larvæ entered the leaf, but the writer did not succeed in rearing adults. A large number of adults of other species were reared from larvæ mining the leaves of garden beets (*Beta vulgaris*), spinach (*Spinacia oleracea*) and Swiss chard (*Beta vulgaris* var. *cicla*), as well as many weeds, such as *Chenopodium album*, *Amaranthus retroflexus*, and *Atriplex patula;* but P. *calyptrata* was not obtained from any of these.

A number of experiments were performed to induce the larvæ of *P. calyptrata* to mine in the leaves of other plants. Three eggs were carefully removed from a dock leaf and placed on a beet leaf. Two days later the eggs hatched, but the larvæ died without entering the leaf. In a second experiment four second-stage larvæ were dissected from *R. obtusifolius* and placed on *Chenopodium album*. The following day the four larvæ were found dead. In a third experiment two third-stage larvæ from *R. crispus* were placed on *C. album*. The following day one larva had entered the leaf and formed a small blotch mine, but the next day the larva died. These experiments seem to substantiate the fact that *P. calyptrata* mines solely in *Rumex* spp.

LIFE HISTORY AND HABITS

EGGS.—The eggs are glossy white, and in the field are laid usually in groups of three to five, occasionally in groups of six or seven, but

[1] STEIN, P. DIE MIR BEKANNTEN EUROPÄISCHEN PEGOMYIA-ARTEN. *In* Wiener Ent. Ztschr., Jahrg. 25, Heft 2/3/4, p. 47–107. 1906.

[2] ZETTERSTEDT, J. W. DIPTERA SCANDINAVIÆ. V. 5, p. 1775, 1846; v. 12, p. 4751, 1855. Lundae.

[3] STEIN, P. NORDAMERIKANISCHE ANTHOMYIDEN ... *In* Berlin. Ent. Ztschr., Bd. 42 (1897), Heft 3/4 p. 239–241, 286. 1898.

seldom singly. They are laid in neat transverse rows on the undersurface of the leaf. In captivity, however, the eggs are scattered over both surfaces of the leaf and are frequently laid singly instead of in groups.

The number of eggs occurring on a single leaf is surprising. As a rule, one finds only 5 or 6 groups, but it is not uncommon to find more. In one instance the writer found 20 groups of eggs on a single leaf, 65 eggs in all. On another leaf, 16 inches long, he found 18 groups of eggs, 47 eggs in all. It is interesting to note that all the larvæ from these eggs did not mature within the leaf on which they were laid, but migrated and started new mines on other leaves. The length of the egg stage is given in Table I.

TABLE I.—*Incubation period of the eggs of Pegomyia calyptrata*

Experiment No.	Eggs laid	Eggs hatched	Length of egg stage
			Days.
A112	May 7	May 13	6
A111	May 8	May 14	6
A113	...do...	do...	6
A117	May 15	May 20	5
A122	May 16	May 22	6
A129	May 23	May 25	2
A132	May 26	May 30	4
A116	May 14	May 19	5
A5	...do.	May 17	3
A134	May 27	May 30	3
A141	May 31	June 5	5
A15	June 23	June 26	3

LARVÆ.—The eggs hatch in from two to six days, and the young larvæ immediately enter the leaf, making small holes through the lower epidermis. All the eggs of a single group hatch at the same time, and the larvæ feed in a common mine, which is at first linear. The larvæ mine side by side, progressing only in a forward direction. They keep close together, and all change their direction of mining at the same time, leaving behind them a short linear path (Pl. 28, D). In about a day, although no definite time can be set, the larvæ begin to enlarge their mine laterally, forming a blotch. They still remain in a common mine, but separate in different directions. It is not an unusual sight to see several such blotches on a leaf. Each represents a number of larvæ that have hatched from a single group of eggs. These increase in size until they interfere with each other, and a large blotch is produced, covering the entire area of the leaf. Many of the larvæ are naturally forced to abandon their mines and form new ones in other leaves. The presence of nearly mature larvæ in small blotch mines is an indication that they have entered fresh leaves (Pl. 28, H).

The writer has removed a larva from its mine and watched it form a new one. At first the larva cuts a short slit in the epidermis of the leaf. Then by inserting the mouth hooks in this slit and working them back and forth the lower and upper epidermises are separated. The larva then pushes the anterior end of its body into the small opening which it has made. After the first two segments have been forced into the leaf, it is only a matter of a few minutes before the larva works its way completely within it. This operation is accomplished with many vigorous twists of the body as it is drawn into the leaf. Larvæ of the third instar bury themselves completely in the leaf in less than 20 minutes.

The feeding habits of the larvæ are most interesting and can be very conveniently watched under a microscope by means of transmitted light. The pharyngeal skeleton (fig. 1, A–D) bearing the mouth hooks, is loosely joined within the first and second segments and is capable of great freedom of movement. These move very rapidly when the larva is feeding and are very effective tools for tearing away the parenchyma of the leaf. There are two distinct types of movement which the mouth hooks possess. First, a lateral movement; the mouth hooks are turned perpendicular to their normal position and work in the plane of the mine. They strike to the right for a short time, then to the left, separating the upper and lower epidermises but not tearing away any of the tissue. The second movement of the hooks is a vertical one. As the hooks are held in their normal position, they are thrust downward, and pieces of the parenchyma are torn loose from the bottom of the mine. In the first case the two epidermal layers are only separated, while in the second the parenchyma of the leaf is actually removed.

TABLE II. *Length of the larval period of Pegomyia calyptrata*

Experiment No.	Eggs hatched.	Puparium formed.	Length of larval period.
			Days.
A 126	May 23	June 7	15
A 129	May 25	June 10	16
A 135	May 29	June 8	10
A 35	July 3	July 12	9
A 225	Oct. 8	Oct. 21	13

From Table II it will be seen that the larval period varies somewhat. This variation is undoubtedly due to weather conditions. During warm weather the larvæ mature rapidly, but during cooler weather the miners become inactive, and the larval period may be prolonged several days. Unfortunately the table is not complete enough to show this. However, it shows a tendency for a longer larval period in early May and October than in the latter part of May and July.

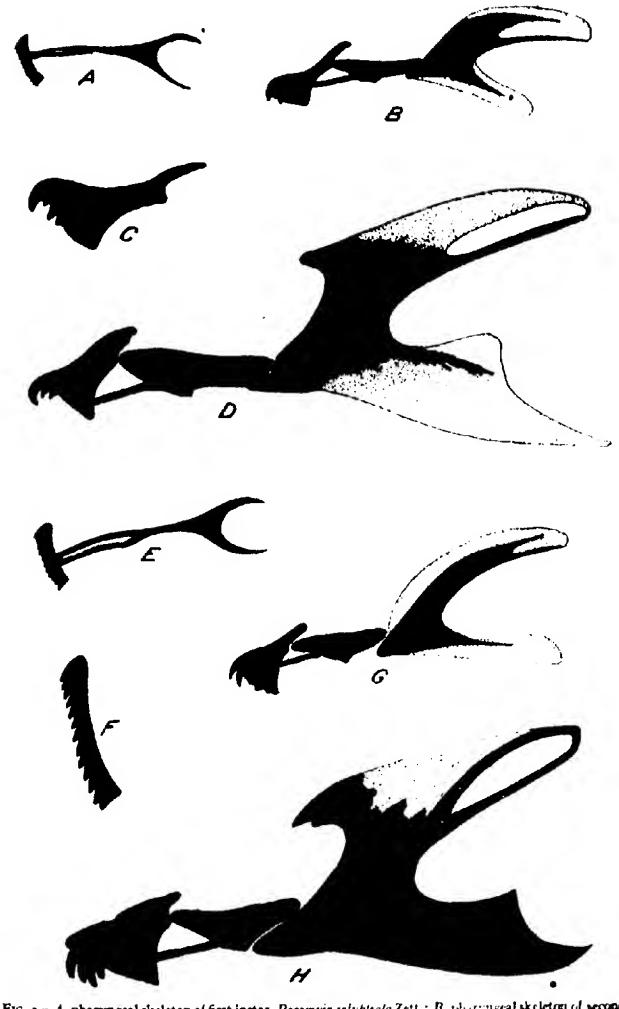


FIG. 1.—*A*, pharyngeal skeleton of first instar, *Pegomyia calyptrata* Zett ; *B*, pharyngeal skeleton of second instar, *P. calyptrata; C*, mandibular sclerite of second instar, *P. calyptrata: D*, pharyngeal skeleton of third instar, *P calyptrata; E*, pharyngeal skeleton of first instar, *P. affinis* Stein ; *F*, mandibular sclerite of first instar, *P. affinis; G*, pharyngeal skeleton of second instar, *P. affinis; H*, pharyngeal skeleton of third instar, *P. affinis*.

FORMATION OF THE PUPARIA.—The mature larvæ escape through the epidermis of the leaf, but seldom make their exit through a definite hole. Usually the upper epidermis become dry and parchment-like, rupturing itself and allowing the larvæ to escape. Sometimes the larvæ cut circular holes through the epidermis. They fall to the ground and ordinarily penetrate the soil to a depth of 2 or 3 inches. If the ground is hard, they do not enter, but form their puparia beneath leaves or other rubbish. The depth to which the larvæ penetrate varies considerably. To determine this, a few experiments were performed with full-grown larvæ. For this purpose a root cage was used, having a space of ¼ inch between the glass and the back. This space was filled with loose sandy soil. The surface of the earth was covered to prevent the larvæ from escaping. This darkened the top of the soil and slightly altered normal conditions.

Twenty-seven mature larvæ were placed on the surface of the soil. The following day eight puparia were found on top of the earth. The remainder of the larvæ either failed to transform or escaped. In a second experiment 32 mature larvæ were placed on the surface of the soil. Four larvæ transformed on the surface, six were found at a depth of 2 inches, one at a depth of 5 inches, and two at a depth of 6 inches. These experiments, though few in number, give some idea of the depth to which the larvæ penetrate loosely compacted soil.

In captivity many of the larvæ formed their puparia within the leaves. Mined leaves were collected in large numbers and put in receptacles to obtain puparia. In most cases the puparia were found in the leaves or between the leaves from which they had issued. A few of the larvæ wandered about in an attempt to find a more suitable place in which to transform. Evidently many of the larvæ under natural conditions never enter the soil.

ADULTS.—While working with the adults, the writer had an opportunity to experiment with several types of rearing cages. A cage similar to the Riley cage, covered with cheesecloth on three sides and with glass on the fourth, gave good results when large numbers of individuals were used, but proved useless for individual pairs of flies. Glass cylinder cages (Pl. 29, B) yielded better results for this purpose, but were unsatisfactory because the adults escaped when the cylinders were lifted. The most satisfactory type of cage proved to be the Fiske cage (Pl. 29, A, C), used a great deal in the rearing of parasites by the United States Department of Agriculture at Melrose Highlands, Massachusetts. The small opening at the top readily permits one to introduce food, while the ring at the bottom prevents the flies from escaping.

In spite of all the precautions in handling and feeding the adults, they did not live long in captivity. One male was kept alive for 18 days. Table III gives the length of life of the males and females as obtained from pairs that were kept in captivity.

TABLE III.—*Length of the life of the adults of Pegomyia calyptrata*

Females.			Males		
Issued.	Died.	Lived	Issued	Died	Lived
		Days			Days
July 2	July 5	3	July 2	July 5	3
July 3	July 19	16	July 3	July 21	18
July 19	July 20	1			
July 20	July 27	7			
Aug. 16	Aug. 23	7	Aug. 16	Aug. 23	7
Do.	Aug. 24	8	Do.	Aug. 22	6
Aug. 22	Sept. 2	11	Aug. 22	Sept. 2	11
Aug. 26	Aug. 30	4	Aug. 26	Aug. 30	4
Aug. 31	Sept. 10	10	Aug. 31	Sept. 7	7
Sept. 1	Sept. 3	2	Sept. 1	Sept. 3	2
Sept. 23	Sept. 28	5			
Oct. 10	Oct. 12	2	Oct. 10	Oct. 12	2
May 6	May 11	5			
			Aug. 16	Aug. 22	6

Copulation frequently takes place a few hours after the female issues. Flies that had issued during the night were observed in several cases to copulate during the following morning. In order to induce copulation, good results were obtained by placing pairs in 6 dram vials. They could be conveniently watched, and mating took place quite readily. Pairs that were in larger cages usually died before copulation took place. Copulation usually lasted about 40 minutes; in one case it lasted for 1 hour and 15 minutes. It is still unknown whether a second or later copulations take place.

The number of eggs laid was obtained only by dissection. About 62 ripe eggs were found in a fertilized female. All these are evidently laid during one oviposition period.

NUMBER OF GENERATIONS.—The writer has been unable to determine accurately the number of generations a year. This is due to the repeated difficulty he experienced in attempts to keep the adults alive for any considerable period of time, as well as the failure to obtain females to oviposit. Only four of many females experimented with laid eggs, and of these none laid more than five eggs. Under such conditions it was impossible to rear the generations through from eggs to adults. However, the number of generations was followed quite accurately by observing conditions in the field and comparing them with conditions under observation at the insectary.

The various generations overlap one another, and it is impossible to tell, from outside conditions alone, when one ends and the other begins. The terminations of the first generation can be plainly seen. For example, in the spring of 1916 the first flies to issue from puparia kept out of doors over the winter appeared on May 4. The adults continued to issue until May 16. The females began laying soon after emerging;

those issuing from puparia in captivity laid in four or five days. This checks with condition in the field, for the first eggs were observed outside on May 7. The larvæ matured in from 9 to 16 days. Toward the end of the larval period they mined rapidly, producing conspicuous blotches on the leaves. The maturing of the first-generation larvæ could thus be easily distinguished. After this it was impossible to follow the number of generations by observation in the field alone. One can go out in the field any time during the summer and find eggs, larvæ, puparia, and adults all at the same time.

From a number of eggs laid in the spring by the females, overwintering as puparia, the writer succeeded in rearing adults of the first generation. These adults laid a few eggs, but none of them hatched. By bringing in eggs from the field at this time the writer reared adults of the second generation. This was continued through the summer, and a fair idea of the number of generations was obtained. During 1915 and 1916 the writer obtained three and a partial fourth generation. The majority of the fourth generation were overtaken by cold weather and perished.

From Table IV it will be seen that some of the puparia formed in June and July, as well as those formed in September, did not give forth adults the same year that they were formed, but overwintered as puparia, and the adults issued the following spring. This seems to be a provision of nature to insure the continuation of the race the following year in case all the individuals of any generation should perish.

TABLE IV.—*Length of the pupal stage of Pegomyia calyptrata*

Experiment No.	Number of puparia	Puparia formed.	Adults issued.	Number of adults.	Length of stage.
					Days.
A150	6	June 5	July 1	1 ♀	26
A168	4	June 9	July 5	2 ♀	26
		do	July 6	1 ♀	27
A165	29	do	July 1	6 ♂	22
		do	July 3	1 ♀	24
A9	4	June 11	July 6	1 ♂	25
A14	10	do	July 3	4 ♂	22
		do	July 4	3 ♀	23
A167	29	June 13	July 3	1 ♂	20
A169	10	do	July 5	1 ♀	22
A182	17	June 16	July 3	1 ♂ 3 ♀	18
A17	3	June 25	July 16	1 ♂	21
		do	May 6[a]	1 ♀	317
A27	3	July 16	Aug. 5	3 ♂	20
A26	5	July 17	Aug. 6	1 ♂	20
		do	Aug. 8	1 ♂	22
A28	8	July 23	Aug. 13	1 ♂	21
		do	Aug. 14	1 ♂	22
		do	May 4[a]	1 ♀	288
A31	6	July 27	Aug. 14	2 ♂	18
		do	Aug. 16	1 ♀	20
		do	May 5[a]	1 ♀	284

[a] Adults issued the following year from puparia kept out of doors over the winter.

TABLE IV.—*Length of the pupal stage of Pegomyia calyptrata*—Continued

Experiment No.	Number of puparia	Puparia formed	Adults issued	Number of adults	Length of stage
					Days
A32	17	July 29	Aug. 15	1 ♂	17
		do	Aug. 16	1 ♀	18
		do	Aug. 18	2 ♂	20
A34	4	July 30	Aug. 17	1 ♀	18
A36	11	Aug. 1	Aug. 25	1 ♀	24
A37	9	do	Aug. 20	2 ♂	20
		do	Aug. 21	1 ♀	21
A43	6	Aug. 7	Aug. 30	1 ♀ 1 ♂	23
		do	Aug. 31	1 ♀	24
A188	53	Aug. 9	Aug. 29	1 ♀	20
		do	Aug. 30	1 ♂	21
		do	Sept. 1	1 ♀ 1 ♂	23
A190	18	do	do	1 ♀	24
A52	2	Aug. 12	Aug. 30	1 ♀	18
A60	6	Aug. 21	Sept. 11	1 ♂	21
		do	Sept. 13	1 ♀	23
A74	5	Sept. 2	May 6[a]	1 ♀	248
A77	2	Sept. 3	May 5[a]	1 ♀	246
A78	3	Sept. 4	May 4[a]	1 ♂	244
		do	May 5[a]	1 ♀	245
A93	71	Sept. 17	Oct. 9	2 ♂ 1 ♀	23
		do	Oct. 11	2 ♂ 1 ♀	25
		do	May 4[a]	6 ♂ 2 ♀	235
		do	May 5[a]	3 ♀	236
		do	May 6[a]	1 ♂	237
A97	23	Sept. 29	Feb. 14[b]	1 ♂	138[b]
		Oct. 10	May 4[a]	1 ♂	209
A102	16	do	May 5[a]	2 ♀	210
		do	May 6[a]	1 ♀	211
		do	May 7[a]	1 ♀	212
A107		Oct. 18	May 3[a]	1 ♂	201
		do	May 13[a]	1 ♂	208
		do	May 16[a]	1 ♂	213

[a] Adults issued the following year from puparia kept out of doors over the winter.
[b] Adults issued the following year from puparia kept indoors over the winter.

AN UNUSUAL OCCURRENCE WITH ADULTS.—Twice while working with the adults the writer observed them to issue feet first from the puparium (Pl. 28, F). In one case the fly succeeded in freeing itself from the puparium, but the wings never expanded normally. In the second the feet broke through the puparium, but the fly did not succeed in working itself free. It will be noticed from the illustration that the fly's feet are extending from the posterior end of the puparium, showing that the pupa was formed in its normal position within the puparium.

PREDACIOUS AND PARASITIC ENEMIES

The writer reared two parasites from the puparia of *P. calyptrata*, *Opius quebecensis* Prov., and *Dacnusa scaptomyzae* Gahan. These were kindly determined by Mr. A. B. Gahan, of the Bureau of Entomology, United States Department of Agriculture. Several times the writer observed the latter parasite ovipositing in the larva of the miner.

When the parasite alights on the mine, the larva becomes uneasy and gives several twists in an attempt to avoid the attack. Unfortunately the larva is pinned between the two epidermises of the leaf and has little freedom of movement. Thus, it is a simple matter for the parasite to insert its ovipositor into the larva. The parasites transform within the puparia and issue somewhat later than the flies themselves.

A third parasite was reared from the eggs of *P. calyptrata* and determined by Mr. A. A. Girault as *Trichogramma minutum* Riley. Six parasites issued from three eggs, indicating that more than one parasite develops within a single egg.

In addition to the parasites mentioned above, a larva was attacked by an adult of *Nabis ferus* (L.). The writer also caught a nymph of this species with its beak inserted into the larva of the leaf miner, sucking out the juices from its body. *Nabis ferus* is a predacious species and has been known to attack the larva of *Pegomyia hyoscyami* Panz., the spinach leaf miner.

DESCRIPTION OF THE STAGES OF PEGOMYIA CALYPTRATA

EGG.—The egg (Pl. 28, C) is a dirty white, glossy, elongate, and nearly cylindrical in shape. It has a reticulated surface composed of polygonal areas. The micropyle end is rounded or slightly flattened. The opposite end is distinctly pointed. Length 1.18 mm.; width 0.35 mm.

FIRST-STAGE LARVA.—The newly hatched larva measures 1.3 mm. It is creamy white in color and more or less transparent. The trachea and alimentary canal are visible through the integument for the whole length of the body. The first-stage larva possesses the same number of segments as the mature larva, 12 visible segments, but the segmentation is not distinct. The body is rather smooth, except for the minute fleshy locomotory spines, which are located on the intersegmental areas and the margins of each segment posterior to the second. These fleshy spines are arranged in discontinuous thickly set rows encircling the body slightly oblique to the edges of the segments. The first segment, "pseudo-cephalon" (Henneguy) (Pl. 30, E), bears a pair of sensory papillæ a short distance in front of the mouth opening. On each side of the "pseudo-cephalon" there is a row of 12 to 13 minute button-like areas which extend dorsally from the mouth opening. The pharyngeal skeleton is slender and not as highly chitinized as in the later stages. The mandibular sclerite is elongate and serrated, having about 12 sharp teeth. The anterior spiracles are closed, while the posterior spiracles have single breathing pores. The posterior ends of the trachea are slightly chitinized and appear as two parallel chitinized bars at the posterior end of the larva and are more conspicuous than the spiracles themselves.

SECOND-STAGE LARVA.—The segmentation in the second-stage larva is more conspicuous. The alimentary canal and trachea become obscured by the accumulation of fat and the larva becomes more yellowish in color. The "pseudo-cephalon," as in the previous stage, bears a pair of sensory papillæ. In addition, it has a pair of two segmented antennæ which are located slightly posterior to the papillæ and are not as closely approximated as the latter. The pharyngeal skeleton is stronger and more highly chitinized. The mandibular sclerite is triangular and bears four teeth. The button-like areas on the side of the "pseudo-cephalon" are present but reduced to five in number. Similar to the first-stage larva the intersegmental areas and edges of the segments posterior to the second are encircled by several discontinuous thickly set rows of minute fleshy locomotory spines. These become less pronounced posteriorly. There is an anterior spiracle on each side inserted between the second and third body segments. Each spiracle has from 24 to 26 breathing pores arranged about an oval slightly chitinized, flattened disk. The posterior spiracles project slightly and have two narrow breathing pores. The anus opens between two triangular plates on the venter of the last segment.

THIRD-STAGE LARVA.—When mature the third-stage larva measures from 9 to 9.5 mm. It is yellowish in color, distinctly shiny, and headlike in shape, especially when viewed from above. There is a reddish area within the anterior end of the larva which disappears when the larva is preserved in alcohol. The "pseudo cephalon," as before, bears in front of the mouth opening a pair of two jointed antennæ, and in front of these a pair of sensory papillæ. Five minute button like areas are also present on the side of the "pseudo-cephalon." The pharyngeal skeleton is even more highly chitinized than in the second stage larva. The mandibular sclerite resembles that of the second stage larva, but bears three teeth. The intersegmental areas and edges of the third to the last segment are encircled with minute fleshy locomotory spines as in the previous stages. The posterior spiracles are borne on short tubercles and have three breathing pores. The anus opens between two triangular plates (Pl. 30, D). Table V gives the distinguishing characters of the three larval stages.

PUPARIUM.—The puparium when first formed is yellowish with a reddish area at the anterior end similar to that found in the larva. The colored area soon disappears, and in about two hours the puparium becomes reddish brown in color. The divisions of the segments are marked by a grayish powdery substance. The surface of the segments are marked by fine annular striæ. The triangular anal plates of the larva are visible in the puparium. The adult in issuing breaks a piece from the anterior dorsal portion of the puparium, including the first, second, third, and part of the fourth segments. The anterior spiracles of the larva are removed with the cap, which is broken loose. The mouth hooks of the

third-stage larva can then be seen attached to the inner ventral wall of the puparium.

TABLE V.—*Distinguishing characters of the larval stages of Pegomyia calyptrata*

Characters	First stage.	Second stage.	Third stage.
Reddish area at anterior end.	Absent	Absent	Present.
Sensory papillæ	do	Present	Do.
Button-like areas on side of "pseudocephalon."	12 to 13	5 (Pl. 30, E)	5.
Pharyngeal skeleton	Slender, weakly chitinized.	Stout, strongly chitinized.	Stout, strongly chitinized.
Mandibular sclerite	Elongate, serrated, 12 teeth.	Triangular with long basal projection, 4 teeth.	Triangular, basal projection short, 3 teeth.
Anal spiracle	One breathing pore.	Two breathing pores.	Three breathing pores.

THE LESS COMMON SPECIES, PEGOMYIA AFFINIS

HISTORICAL REVIEW

This species has almost escaped the keen eye of the systematist. It was described by Stein (1897)[1] as *Pegomyia vicina* Lintner. Later he noticed his mistake and in the same paper [1] named the new species "*Pegomyia affinis*." Since that time there has been no reference to this species. The writer describes for the first time the habits of this interesting anthomyid.

DISTRIBUTION

Little or nothing is known about the distribution of *P. affinis*. Stein[1] records it rather abundant in Pennsylvania, Vermont, and Illinois. The writer has reared and collected specimens at Ithaca and Florida, N. Y., and Arendtsville, Pa.

HOST PLANTS

P. affinis, mines exclusively on *Rumex* spp. Adults have been reared from *R. crispus* and *R. obtusifolius*. It is possible that other species of Rumex are attacked.

LIFE HISTORY

EGGS.—The eggs are much more beautiful than those of *P. calyptrata* and are pure white in color. They are laid in neat transverse rows of three to five, rarely six or seven, on the under surface of the leaf. They have never been found as abundant as those of the preceding species and never more than three or four groups have been found on a single leaf. The incubation period is given in Table VI.

[1] STEIN, P. NORDAMERIKANISCHE ANTHOMYIDEN. *In* Berlin. Ent. Zeitschr., Jahrg. 42, p. 239-241, 286, 1897.

TABLE VI.—*Incubation period of the eggs of Pegomyia affinis*

Experiment No.	Eggs laid	Eggs hatched	Length of stage
			Days.
A115	May 11	May 16	5
A119	May 14	May 21	7
A125	May 18	May 24	6
A130	May 24	May 28	4
A131	May 28	do	3

LARVA.—The eggs hatch in from three to seven days. All the eggs of a single group hatch at the same time, and the young larvæ feed together in a common mine. At first this is linear, but soon the larvæ separate in different directions and a blotch mine is formed which obscures the original linear track. The mines produced on the leaves can not be distinguished from those of P. *calyptrata*.

TABLE VII.—*Length of the larval period of Pegomyia affinis*

Experiment No.	Eggs hatched	Puparium formed	Length of larval period
			Days.
A2	May 11	May 23	12
A124	May 21	June 6	16
A124	do	June 8	18
A127	May 22	June 7	16
A143	May 31	June 13	13

From Table VII it will be seen that the length of the larval period varies from 12 to 18 days. A larger number of records would, perhaps, show even greater variation. This variation is due, as in case of *P. calyptrata*, to weather conditions.

The mature larvæ escape from the leaves through the cracked surface of the dried and parchment like mine. If the ground is not too hard, they penetrate to a depth of 2 or 3 inches; otherwise, they form their puparia beneath leaves or rubbish.

NUMBER OF GENERATIONS.—The writer is uncertain as to the number of generations. At first he confused the two species mining dock and thought there was but one. Later he noticed his mistake, but it was then too late to make definite observations. The few notes made seem to indicate that there are but two generations a year. A portion of the first-generation adults issued in from 12 to 18 days; the rest overwintered as puparia and issued the following spring. It is believed that all of the second generation overwinter as puparia and issue the following spring. This seems to be the case, because the eggs and larvæ of this species were not found after the end of June.

Table VIII shows the tendency of the first generation to produce some puparia from which adults issue the same year and others that overwinter and from which adults issue the following year. The species is like *P. calyptrata* in this respect.

TABLE VIII.—*Tendency of individuals of the first generation to overwinter as puparia*

Experiment No	Number of puparia.	Puparium formed.	Adults issued.	Number of adults.	Number of days.
A13	4	June 9	July 3	1 ♂	24
A11	2	...do.....	May 6[a]	2 ♀	333[a]
A10	2	...do.....	July 3	1 ♂	24
A12	9	June 11	May 16[a]	1 ♂	342[a]
A16	4	June 24	May 6[a]	1 ♂ 2 ♀	319[a]
A19	4	July 1	...do.....	3 ♀	313[a]

[a] Overwintered as puparia and issued the following spring

DESCRIPTION OF STAGES

EGG.—The egg is pure white, waxy, and elongate, with a delicate reticulated surface. This reticulation consists of rectangular areas arranged about the egg in parallel longitudinal rows, giving the egg a very regular and beautiful appearance. The micropyle end is slightly flattened. The opposite end is rounded. Length 1 mm.; width 0.35 mm.

FIRST-STAGE LARVA.—The newly hatched larva measures about 1 mm. It is translucent white in color, and the trachea and alimentary canal are visible through the integument for the entire length of the body. The body is decidedly smooth with the exception of the intersegmental areas and the posterior aspect of the last segment which are covered with many transverse rows of fleshy locomotory spines. Anterior to the mouth opening there are a pair of sensory papillæ. The tubercles on the posterior aspect of the last segment are not distinct. The posterior spiracles are borne on short stalks and have single breathing pores.

SECOND-STAGE LARVA.—At first the trachea and alimentary canal are visible through the integument, but soon they become obscured by the accumulation of fat. The whole body is minutely roughened, especially so on the intersegmental areas and the edges of some of the segments. This roughening is caused by transverse rows of minute fleshy locomotory spines, as described in the first-stage larva. The pharyngeal skeleton is strongly chitinized, and the mandibular sclerite bears two well-defined teeth and several smaller ones. Anterior to the mouth opening are a pair of two-jointed antennæ, and in front of these a pair of sensory papillæ. On each side of the "pseudo-cephalon" are a row of minute button-like areas which extend from the mouth opening dorsad. The posterior spiracles have two breathing pores.

THIRD-STAGE LARVA.—The third-stage larva resembles very much the previous stage, but is a dirty white in color. The locomotory spines on the intersegmental areas are more conspicuous than in the second-stage larva. They are usually rendered visible to the naked eye by means of the dirt which catches between them. As in the previous stage the "pseudo-cephalon" bears a pair of two-jointed antennæ and a pair of sensory papillæ in front of the mouth cavity. The anterior spiracles consist of oval slightly chitinized disks surrounded by about 24 breathing pores. On the posterior aspect of the last segment are six small tubercles surrounding the spiracles in a semicircle on the ventral side. The posterior spiracles have three breathing pores.

PUPARIUM.—The puparium is white or slightly creamy in color when first formed, but in about two hours it turns dark brown or almost black. When the puparium becomes dry, it turns grayish in color. The two anal plates of the larva are visible in the puparium and are surrounded by a ring.

COMPARISON OF THE TWO SPECIES

A comparison of the characters of the two species is given in Table IX.

TABLE IX.—*Comparison of the characters of Pegomyia calyptrata and P. affinis*

Characters.	*P. calyptrata*	*P. affinis*
EGGS.		
Color	White, glossy	White, waxy.
Shape	Pointed at one end	Rounded at both ends.
Reticulations	Polygonal	Rectangular
LARVA.		
Color	Yellow, shiny	Dirty white, dull.
Mouth hooks	With three teeth	With four teeth
Sensory papillæ	Close together, smaller than antenna.	Not close together, about same size as antenna.
Posterior end	Not distinctly tuberculate	Distinctly tuberculate.
PUPARIUM.		
Color	Brown	Black
Segmentation	Beadlike	Not beadlike.
ADULT.		
Color	Abdomen yellow, thorax bluish gray.	Abdomen and thorax gray.

PLATE 28

A.—Eggs of *Pegomyia affinis*.

B.—Parasitized eggs of *Pegomyia calyptrata*.

C.—Eggs of *Pegomyia calyptrata*.

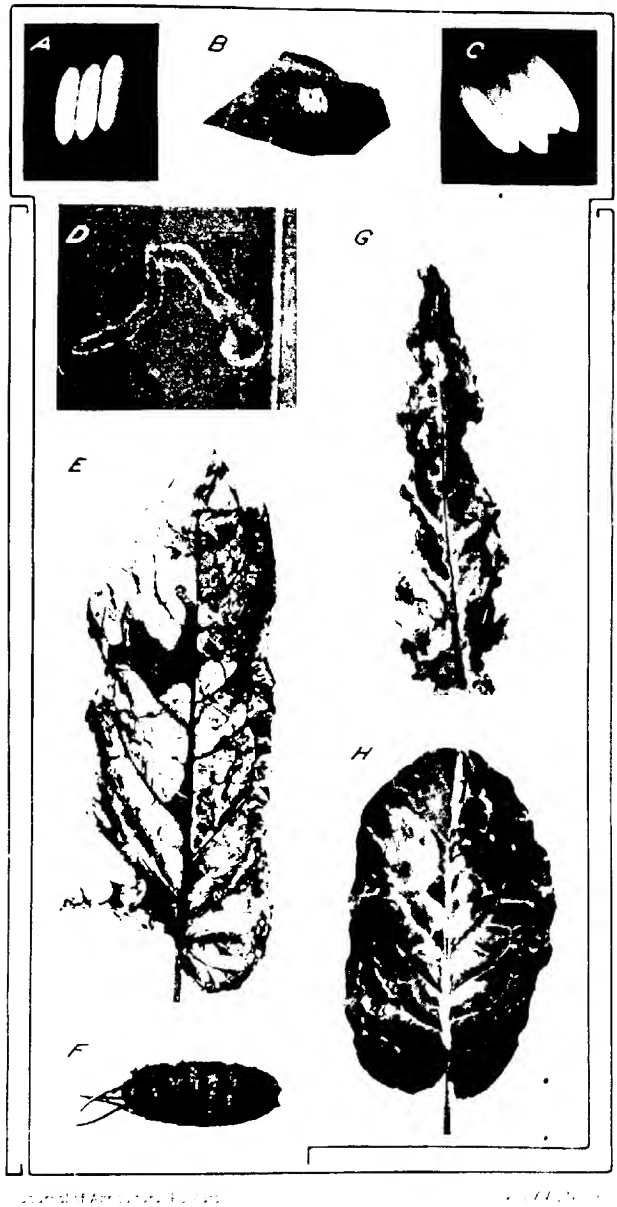
D.—A small mine on Rumex leaf, showing the original linear mine and the beginning of the blotch mine.

E.—A typical mine on *Rumex obtusifolius* produced by the larva of *Pegomyia calyptrata*.

F.—A monstrosity, an adult *P. calyptrata* issuing feet first from its puparium.

G.—A typical mine on *Rumex crispus* produced by the larva of *Pegomyia calyptrata*.

H.—A mine on *Rumex obtusifolius*, produced by a nearly mature larva entering a new leaf to complete its development.


A
B
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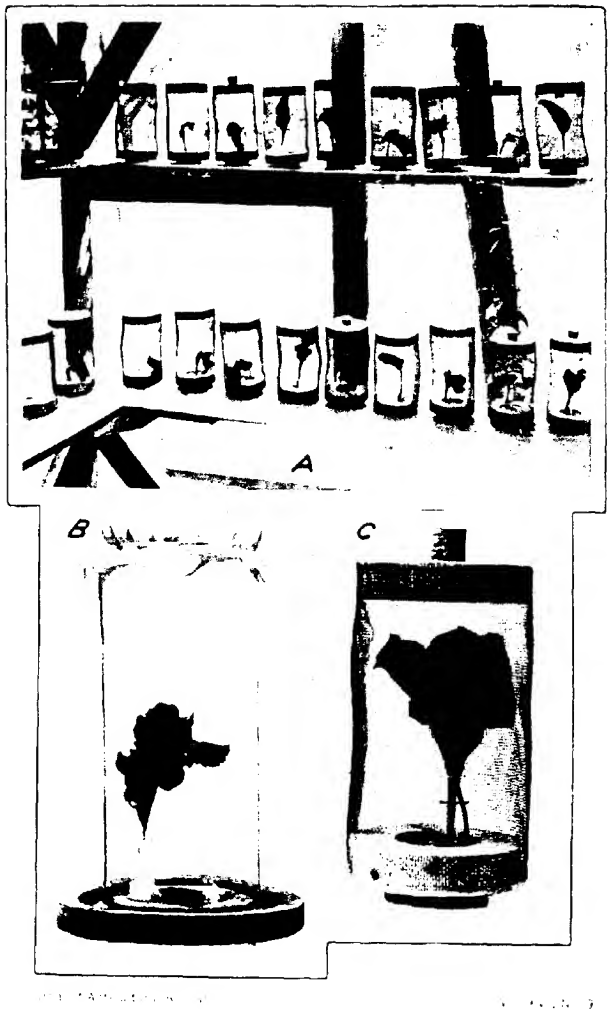
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PLATE 29

A.—Fiske cages, used in studying the adult flies of *Pegomyia* spp., showing the arrangement in outdoor insectary.

B.—Glass cylinder cage, used in studying habits of adult flies.

C.—Fiske cage, used in studying habits of adult flies.

PLATE 30

A.—Ventral aspect of posterior segment of larva of *Pegomyia affinis*, showing lobes and anal opening.

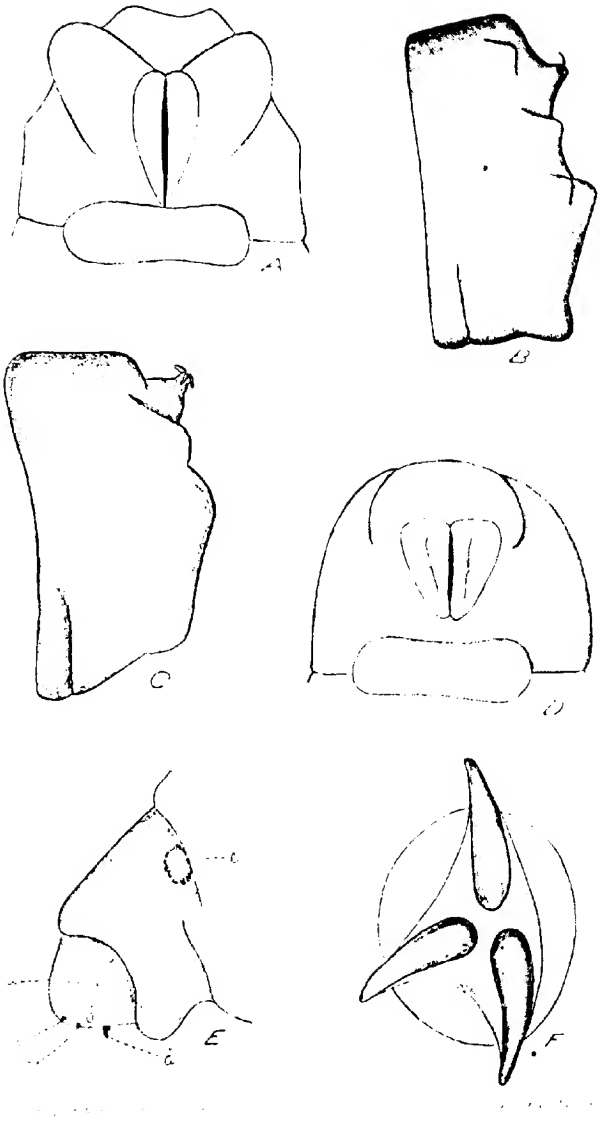
B.—Lateral aspect of posterior segment of larva of *P. affinis*, showing tubercles.

C.—Lateral aspect of posterior segment of larva of *P. calyptrata*.

D.—Ventral aspect of posterior segment of larva of *P. calyptrata*, showing lobes and anal opening.

E.—"Pseudo-cephalon" (Henneguy) of *P. calyptrata*: *a*, button-like areas referred to in text; *b*, sensory papilla; *c*, antenna; *d*, mandibular sclerite; *e*, anterior spiracle.

F.—Posterior spiracle of larva of *P. calyptrata*.


A
B
C
D
E
F

INFLUENCE OF FOREIGN POLLEN ON THE DEVELOPMENT OF VANILLA FRUITS

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INTRODUCTION

The Agricultural Experiment Station at Mayaguez, Porto Rico, has been conducting experiments for a number of years with a view to the establishment of vanilla growing on a commercial scale in Porto Rico. Material representing many species of Vanilla has been obtained, and the adaptation of these species to Porto Rican conditions is being studied. The questions of pollination, fertilization, yield, and character of the finished product have been given attention. The two types of economic value are *Vanilla planifolia*, a plant having a slender pod of high quality, and the "vanillon" type, which has a shorter, thicker pod greatly inferior in quality and market value to the pod of *V. planifolia*, but which possesses a marked advantage in the ease with which it may be cured, since on ripening it does not split open if allowed to remain several days beyond a certain point in ripening which is difficult to determine, as the change in color is slight.

Since the production of hybrids presented alluring possibilities, various reciprocal crosses were made with a view to the development of valuable strains. It soon appeared that these fruits of these hybrids were noticeably different from the others on the same vines. In order to study this phenomenon, numerous additional crosses were subsequently made. The data presented below show an immediate influence of the foreign pollen on the form of the fruits.

Only a very small percentage of vanilla blossoms are pollinated by natural means in Porto Rico. In a series of blossoms of *V. planifolia* under observation by the writer at the Porto Rico Experiment Station only 1.5 per cent of the blossoms were so pollinated.

In hand pollination the usual method and by far the simplest is to use the pollen of the same flower. Where pollen from another flower of the same species has been applied, no resultant change in the form of the fruit produced has been observed.

The typical well-developed fruit of *V. planifolia* from a close-fertilized blossom is a long slender capsule tapering at the stem end but carrying its fullness well down toward the blossom end. It contains thousands of tiny, oily, black seeds.

[1] The writer wishes to acknowledge his indebtedness to Dr. H. J. Webber, Dean of the Graduate School of Tropical Agriculture, University of California, for suggestions.

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The other type of *Vanilla* spp. on which data are here presented is termed "vanillon." Under this name are grouped a number of varieties or species other than *V. planifolia* which, though presenting distinct differences, agree among themselves in having large yellow blossoms in contrast to the much smaller, paler, greenish blossoms of the *V. planifolia*. The fruits are much thicker and shorter than those of the latter and differ from it in being of a more uniform thickness near the two ends, the blossom end frequently being rather tapering.

Where to either the *V. planifolia* or the vanillon stigma pollen of the other has been applied a very decided modification in the form of the fruit has resulted. This modification is in most instances so decided that these fruits can be distinguished from the close-fertilized fruits at a glance.

These conclusions were reached as result of observation and data on crosses made in 1916. Data taken on crosses in 1917 confirmed the work of the preceding season. The accompanying illustrations and tables present the work of the two seasons.

TABLE I.—*Comparison of the girth measurements of the fruit of Vanilla planifolia* ♀ × *V. planifolia* ♂

Length of fruits.	Girth in sixteenths of an inch at 1½ inches from stem end.	Increase or decrease in circumference of pods.	Girth in sixteenths of an inch at 1 inch from blossom end.
Inches.			
6½	22	<	26
6½	18	<	22
7	20	<	24
7	20	<	22
7	22	<	24
7¼	22	<	28
7¼	20	<	25
7½	22	<	24
7½	22	<	25
7¾	23	<	25
7¾	23	<	28
8	22	<	26
8	20	<	24
8½	22	<	28
8½	24	<	28
8¼	20	<	30
8¼	21	<	33
8¾	23	<	30
9	23	<	26
9	24	<	28
9¼	24	<	30
9¼	22	<	28
9¾	22	<	29
Total	501	<	613
Average	21.8	<	26.7

In the tables the measurements of girth are given in sixteenths of an inch expressed as integers for readier comparison. The comparative relation of the two girths is indicated by the symbol between them. "P" indicates *V. planifolia*. "V." indicates species of Vanilla of vanillon type, the accompanying numeral specifying the particular variety. V 13 is a Guadeloupe variety, V 34, V 43, V 51, and V 52 are from Panama, and V 62 is from Mexico.

Table I shows that the typical fruit of *V. planifolia* is considerably greater in girth at 1 inch from the blossom end than at 1½ inches from the stem end. This difference amounted to 22.4 per cent in these fruits. They had been selected to compare in length with the crosses. One hundred unselected fruits which averaged somewhat smaller than these gave the same relative difference of 19 per cent, 93 showing a greater apical girth, with 7 having the two girths equal. The latter is true chiefly in short, poorly developed fruits. These measurements show that the typical, well-developed fruit tapers considerably more at the stem end than at the blossom end.

Table II shows the diametrically opposite development where *V. planifolia* has been fertilized by vanillon pollen. The fruits which developed from the crossed flowers tapered at the blossom end and were well filled at the stem end. The girth at 1½ inches from the stem end measured 25.1 per cent greater than that at 1 inch from the blossom end.

TABLE II. *Comparison of the girth measurements of the fruit of Vanilla planifolia* ♀ × *vanillon* ♂

V × P	Length of fruit in inches.	Girth in sixteenths of an inch at 1½ inches from stem end.	Increase or decrease in circumference of pods	Girth in sixteenths of an inch at 1 inch from blossom end.
V 52	6½	25	>	18
V 34	6¾	26	>	20
V 34	7	27	>	22
V 52	7	24	>	18
V 52	7	26	>	20
V 34	7¼	26	>	20
V 52	7¼	26	>	20
V 52	7½	26	>	20
V 62	7½	28	>	21
V 62	7½	28	>	20
V 52	7¾	29	>	22
V 34	8	30	>	21
V 52	8	27	>	20
V 52	8¼	30	>	24
V 52	8¼	26	>	24
V 52	8¼	30	>	22
V 52	8¼	30	>	28
V 52	9	28	>	26
V 52	9¼	30	>	27
V 52	9¾	32	>	30
Total		554	>	443
Average		27.7	>	22.2

Typical specimens are shown in Plate 31, figures A showing the whole fruits and figure B the same with sections made at the lines of measurements. From left to right the first of the paired fruits shows the development of *V. planifolia* when close-fertilized, while the second shows the results when vanillon pollen has been applied. The sections show that many more ovules have been fertilized near the apex of the ovary by the *V. planifolia* pollen, while the vanillon pollen has fertilized many more near the base than near the apex. This difference in location of the ovules fertilized accounts for the striking difference in form which results from the application of *V. planifolia* or vanillon pollen to the *V. planifolia* stigma.

Table III shows the typical close-pollinated vanillon fruit to be of nearly equal average girth at 1 inch from either end. Of the 54 fruits measured the two girths were equal in 12, the apical girth greater in 18, and the basal girth greater in 24 fruits. The girth at 1 inch from stem end averaged 0.5 per cent greater than at 1 inch from blossom end.

TABLE III.—*Comparison of the girth measurements of the fruit of vanillon ♀ × vanillon ♂*

V	Length of fruit in inches.	Girth in sixteenths of an inch at 1 inch from stem end.	Increase or decrease in circumference of pods.	Girth in sixteenths of an inch at 1 inch from blossom end.
V 51	3	34	>	32
V 13	3 1/16	39	>	36
V 13	3 1/16	37	=	37
V 13	3 1/16	40	=	40
V 34	3 1/16	30	>	29
V 13	3 3/16	41	>	39
V 13	3 3/16	40	=	40
V 51	3 3/16	38	>	36
V 52	3 3/16	40	>	36
V 52	3 3/16	40	>	37
V 52	3 3/16	36	>	34
V 13	4	40	=	40
V 13	4	42	=	42
V 34	4	32	>	31
V 43	4	40	>	32
V 43	4	31	>	29
V 51	4	40	>	36
V 13	4 1/16	42	>	43
V 13	4 1/16	44	>	45
V 13	4 1/16	42	>	43
V 51	4 1/16	41	>	40
V 13	4 1/16	45	=	45
V 13	4 1/16	42	<	43
V 13	4 1/16	43	=	43
V 13	4 1/16	43	<	44
V 52	4 1/16	42	<	38
V 13	4 3/16	46	>	45
V 13	4 3/16	41	>	45
V 52	4 3/16	41	>	40
V 52	5	47	=	47
V 52	5	42	=	42
V 52	5	41	<	44
V 34	5 1/16	35	<	36
V 34	5 1/16	33	=	33
V 43	5 1/16	37	>	34
V 34	5 1/16	35	<	36

TABLE III.—*Comparison of the girth measurements of the fruit of vanillon ♀ × vanillon ♂*—Continued.

V	Length of fruit in inches.	Girth in sixteenths of an inch at 1 inch from stem end.	Increase or decrease in circumference of pods.	Girth in sixteenths of an inch at 1 inch from blossom end.
V 43	5 1/4	43	V	38
V 43	5 1/2	40	V	40
V 34	5 3/4	34	V	30
V 43	5 3/4	42	V	39
V 34	6	35	V	38
V 34	6	31	V	35
V 43	6	40	V	45
V 34	6 1/4	37	V	42
V 43	6 1/4	43	V	41
V 34	6 1/2	32	V	35
V 43	6 1/2	38	V	38
V 43	6 1/2	39	V	38
V 34	6 3/4	29	V	34
V 43	6 3/4	35	V	[a] 42
V 43	6 3/4	44	V	48
V 43	6 3/4	46	V	44
V 43	7	45	V	47
V 43	7 1/4	45	V	43
Total	...	2,126	V	2,115
Average	...	39.4	V	39.2

[a] This fruit which externally resembled a V♀×P♂ cross had ovules fertilized throughout its length which was true of no crosses of V43♀×P♂.

TABLE IV.—*Comparison of the girth measurements of the fruit of vanillon ♀ × Vanilla planifolia ♂*

V ♀	Length of fruit in inches.	Girth in sixteenths of an inch at 1 inch from stem end.	Increase or decrease in circumference of pods.	Girth in sixteenths of an inch at 1 inch from blossom end.
V 51	2 1/4	28	^	33
V 13	3 1/4	33	^	36
V 34	3 1/4	24	^	33
V 13	3 1/2	34	^	39
V 13	3 1/2	34	^	40
V 13	3 3/4	36	^	40
V 13	3 3/4	35	^	42
V 52	3 3/4	38	^	43
V 13	4	37	^	44
V 13	4	27	^	43
V 52	4	41	^	44
V 13	4 1/4	38	^	45
V 43	4 1/2	31	^	45
V 43	4 1/2	29	^	44
V 34	4 3/4	32	^	41
V 43	4 3/4	27	^	40
V 34	5	31	^	43
V 34	5	28	^	41
V 43	5	30	^	43
V 43	5 1/4	35	^	49
V 43	5 1/4	34	^	47
V 62	5 1/4	36	^	40
V 43	5 1/2	35	^	50
Total	...	753	^	965
Average	...	32.7	^	42.0

Table IV shows the decided change in form produced by the application of *V. planifolia* pollen to the vanillon stigma. Without exception the apical girth of the fruits from crossed flowers was greater than the basal girth. The average girth at 1 inch from blossom end was 28.2 per cent greater than that at 1 inch from stem end. Not only was this relative difference evident in the development of the two ends of the crossed fruits but while the development of the base of the crosses fell far below that of the close-fertilized fruits, the development of the apex in the former exceeded that of the latter for the fruits measured.

Plate 32, A, shows V 13 fertilized by *V. planifolia* in the upper row with close-fertilized fruits of V 13 in the lower row. Figure B shows the same fruits with sections cut at the lines of measurement. These sections show the fertilization in the two ends to be much more uniform for the close-fertilized fruits than for the crossed fruits which show a heavier fertilization near the apex than near the base.

In Plate 33, A and B, at the right are shown four fruits of V 34 from blossoms fertilized by *V. planifolia*. The four at the left are from close-fertilized blossoms of V 34. The heavy fertilization of ovules in the apex of the crosses is clearly shown.

In Plate 34, A and B, are shown fruits of V 43, the upper row from blossoms fertilized by *V. planifolia* pollen, the lower row from close-fertilized blossoms. The effect of the *V. planifolia* pollen was more pronounced on this variety than on any other tested, as none of the seven crossed fruits showed any ovules fertilized in the stem end, in some instances none for more than 2 inches from the base, though all showed large numbers of ovules fertilized near the blossom end. All of the close-fertilized fruits examined, however, showed many ovules fertilized near the base, fertilization here being frequently heavier than near the blossom end. In figure B at the top are shown the middle sections of the two fruits at the left in the upper row. The point to which the ovules are fertilized is clearly shown, the ovules appearing as black dots. At the bottom of figure A the middle sections of the two fruits at the left in the lower row show fertilization throughout.

Plate 35, A, shows longitudinal sections of typical fruits, being from left to right V ♀ × V ♂, V ♀ × P ♂, P ♀ × V ♂, and P ♀ × P ♂.

The proportions of the *V. planifolia* and vanillon blossoms suggest a possible reason for the difference in location of the ovules fertilized by the two kinds of pollen with the resultant alteration in the form of the fruit. At blossoming the difference in the length of the ovaries is slight, but the vanillon column is much longer than that of *V. planifolia*, exceeding the length of the latter in some instances by as much as 60 to 70 per cent. Plate 35, B, shows a cleared vanillon column and ovary above with that of *V. planifolia* below, placed to compare the length of ovary at the right and the length of column at the left.

It seems quite reasonable to suppose from the heavy fertilization of ovules near the apex and sparse fertilization or entire absence of fertilization near the base of the ovary when the vanillon stigma has been pollinated with *V. planifolia* pollen that these pollen tubes are unable to reach, or reach in only limited numbers the ovules in the far end of the ovary, which are at a considerably greater distance from the stigma than the farthest ovules of the *V. planifolia* ovary. Even in its own ovary the *V. planifolia* pollen causes a much heavier fertilization near the apex than near the base. This inability of *V. planifolia* pollen tubes to reach the farthest ovules was particularly marked when *V. planifolia* pollen was applied to V 43, which is one of the largest flowered of the vanillon varieties.

The vanillon pollen tubes, however, reach ovules in the *V. planifolia* ovary at a much shorter distance from the stigma than in their own flower. Many of these first ovules which the *V. planifolia* pollen would fertilize are left unfertilized by the vanillon pollen, the pollen tubes passing by to other ovules which are nearer the normal distance from stigma to ovary in the vanillon flower, and causing a much heavier fertilization in the base of the pod than would the *V. planifolia* pollen.

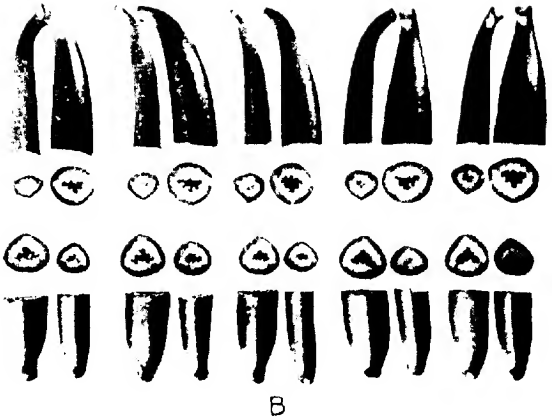
This might possibly indicate in this instance the necessity for a certain maturity of development of the pollen tube before the ovule can be fertilized.

PLATE 31

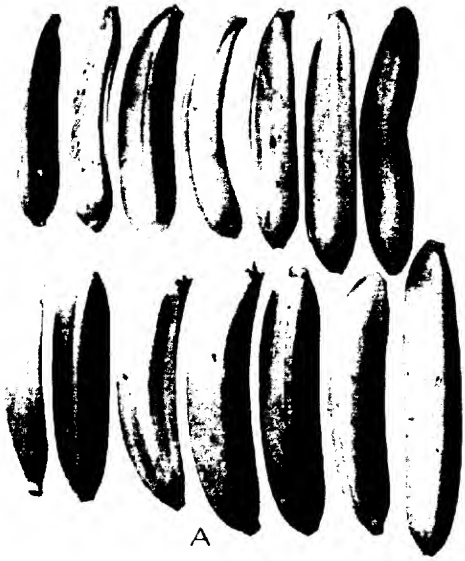
A.—Left to right, *Vanilla planifolia*, first of each pair close-fertilized, second fertilized with vanillon pollen.

B.—Cross sections of same fruits.

A



B



A

B

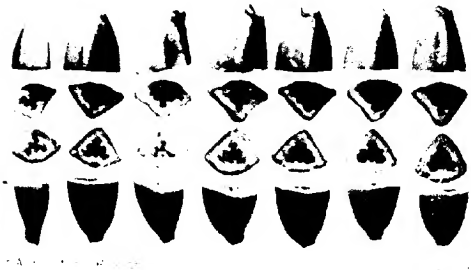


PLATE 32

A.—Vanillon No. 13 fruits, close- and cross-fertilized. Lower row close fertilized, upper fertilized with pollen of *Vanilla planifolia*.

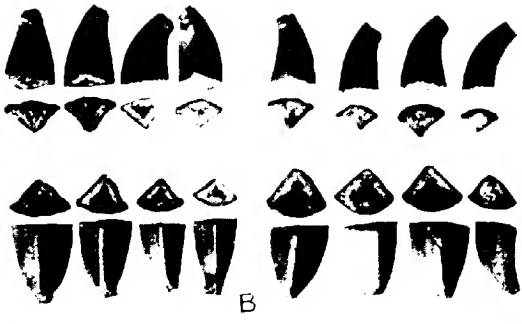
B.—Cross section of same fruits.

PLATE 33

A.—Vanillon No. 34: Fruits at right fertilized with pollen of *Vanilla planifolia*, left close fertilized.

B.—Cross sections of same fruits.

A



B

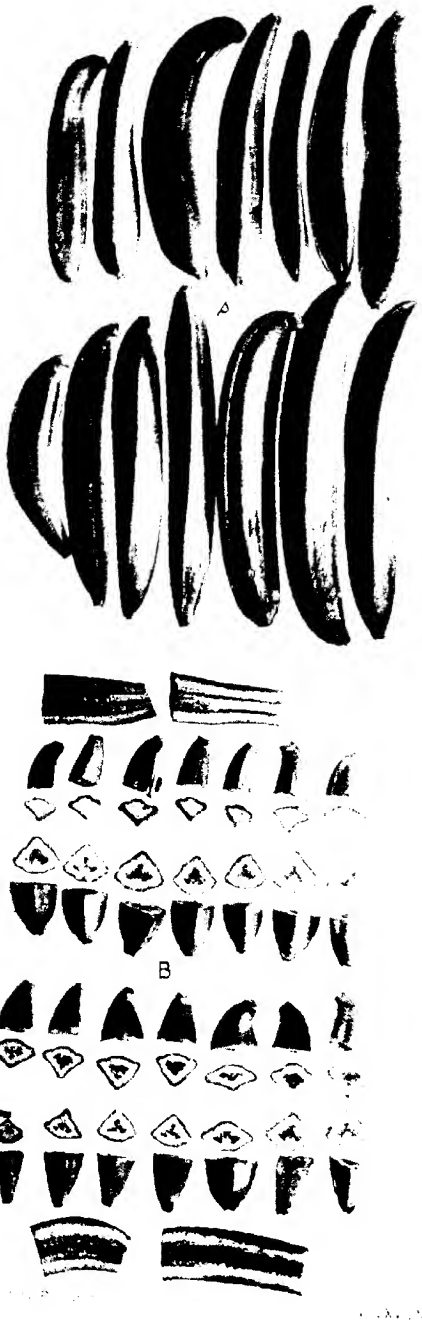

A
B

PLATE 34

A.—Vanillon No. 43 fruits. Upper row fertilized with pollen of *Vanilla planifolia*, lower row close-fertilized.

B.—Cross sections of same fruits.

PLATE 35

A.—Longitudinal sections of vanilla fruits. Left to right: Vanillon, pistillate, × vanillon, staminate; vanillon, pistillate, × *Vanilla planifolia*, staminate; *Vanilla planifolia*, pistillate, × vanillon, staminate; *Vanilla planifolia*, pistillate, × *Vanilla planifolia*, staminate.

B.—Comparative length of cleared columns and ovaries. Vanillon above, *Vanilla planifolia* below.


A
B

A BLOOD-DESTROYING SUBSTANCE IN ASCARIS LUMBRICOIDES

[PRELIMINARY PAPER]

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RELATION OF ASCARIS INFECTION TO ANEMIA

The view that parasitic worms secrete toxic substances which are absorbed by the host and which are responsible to a considerable extent for the symptoms which often accompany parasitic infections, has received strong support from experimental work with the body fluids and extracts of the various species of Ascaris that parasitize man and domesticated animals. Numerous experiments have shown that the body fluids and extracts of these worms produce decidedly harmful effects on susceptible animals into which they are injected. While it must be admitted that the effects of such injections are far more pronounced than the symptoms usually exhibited by animals that are known to harbor in their intestines numerous ascarids, experiments of this nature have thrown considerable light on the pathology of Ascaris infection, and have also added to our knowledge of the metabolic products of these parasitic worms.

That Ascaris may cause anemia is the opinion of various observers. According to Schimmelpfennig (*9*)[1] and Weinberg and Julien (*14*), the post-mortem appearance of horses infected with ascaris suggests a condition of anemia. As far as man is concerned, a number of clinicians have emphasized the importance of *Ascaris lumbricoides* in relation to anemia. Thus, Demme (*3*) reports two cases of grave anemia in children, resembling pernicious anemia, which he attributed entirely to the presence of *A. lumbricoides* in the intestine. In one case death occurred, the cause of the disease not having been recognized, whereas in the second case complete recovery followed anthelmintic treatment. François (*5*) cites a number of cases of severe anemia resembling that of ancylostomiasis, in which *A. lumbricoides* was evidently the causal factor. Guiart (*8*) considers Ascaris to be a causal agent in anemia, ranking close to Dibothriocephalus and Ancylostoma in importance.

[1] Reference is made by number (italic) to "Literature cited," p. 257-258.

In view of the apparent importance of Ascaris as a cause of anemia, the possible relation between the secretions of the parasite [1] and the anemia of the host has a high degree of practical interest. Certain investigators, notably Schimmelpfennig (*9*) and Flury (*4*), attribute hemolytic properties to the body fluids of Ascaris. Flury states, in fact, that the excretions of Ascaris when kept *in vitro* are hemolytic, and inclines to the view that anemia may be caused by the absorption of toxic substances produced by the worms. Weinberg (*12*, *13*), Whipple (*15*), Alessandrini (*1*), and several other investigators, on the other hand, deny the presence of hemolytic substances in Ascaris and state quite emphatically that blood corpuscles of the host in contact with extracts of the worms remain intact. Recently Shimamura and Fujii (*10*), in a report of experiments with extracts of Ascaris on various animals, state that alcoholic and ethereal extracts of the parasites are hemolytic, but that watery extracts of the body substance of the worms previously freed from the ether and alcohol soluble portions produce no effect on red blood cells.

SCOPE AND SUMMARY OF EXPERIMENTAL WORK

For some time past the writer has been studying the problem of the possible absorption of toxic products by animals harboring ascarids. In this work *A. lumbricoides* of swine,[2] of which an abundant supply is easily obtained, has been utilized. The experiments, the results of which are briefly summarized in this paper, were undertaken with a view of determining (1) whether the body fluids of the parasites are hemolytic, (2) whether the excretions of the worms kept *in vitro* contain blood-destroying substances, and (3) the relation which may exist between the anemia of ascariasis and the absorption by the host of toxic substances produced by the parasites. Sufficient data have already been accumulated to warrant certain conclusions, as follows:

(1) The body fluid of *A. lumbricoides* taken from worms shortly after their removal from the host is not hemolytic to the washed erythrocytes of swine, cattle, sheep, rabbits, guinea pigs, and rats.

(2) The fluid from worms which after removal from their host are kept alive in salt solution for a few days acquires hemolytic properties. Fluid from worms kept *in vitro* for 24 hours is only slightly hemolytic if at all, but fluid from worms kept under similar conditions from six to eight days is decidedly destructive to the red blood corpuscles of swine and sheep.

(3) The hemolytic property of the fluid is thermostabile and is not destroyed by boiling.

[1] Several investigators have shown that the fluid and extracts of human, horse, and swine ascaris have indistinguishable chemical and physiological properties.

[2] Ascaris of swine is also referred to as *Ascaris suum* in order to distinguish it from the form which parasitizes man. The two forms are morphologically indistinguishable, however, so far as our present knowledge goes.

(4) There appears to be an inverse relation between the hemolytic property of the fluid and the presence of oxyhemoglobin in it. Fluid from fresh worms contains oxyhemoglobin and is nonhemolytic. When, however, the worms are kept alive *in vitro*, the oxyhemoglobin disappears from the fluid and can no longer be detected by spectroscopic examination one week after the worms have been removed from the host. Meanwhile the fluid becomes hemolytic. Whether oxyhemoglobin in itself is the sole factor in the inhibition of hemolysis or whether other substances are involved which are associated with the oxyhemoglobin and disappear simultaneously with it has not been determined.

(5) Salt-solution extracts of the worms made by grinding up 4 to 10 gms. of the fresh body substance of the parasites and suspending it in 100 cc. of an 0.85 per cent solution of sodium chlorid are hemolytic to the washed erythrocytes of swine and other mammals, the hemolytic potency of the extracts varying directly within certain limits with the duration of the extraction. The reaction is independent of the acidity of the solution, since it is not impaired by neutralization.

(6) Extracts of dried worms in an 0.85 per cent solution of sodium chlorid are decidedly hemolytic to the red corpuscles of various animals.

(7) Salt-solution extracts of the intestine of the worm are more destructive to blood corpuscles than extracts of the body wall, of the reproductive organs, or of the entire worm.

(8) The various salt-solution extracts also do not lose their hemolytic properties on boiling.

(9) The addition of blood serum to tubes containing a mixture of red blood corpuscles and body fluid or extract of the worms usually inhibits hemolysis.

(10) The hemolytic property of the fluid and of extracts of the worms can also be destroyed by the addition of a small quantity of laked blood.

(11) Excretions of the worms absorbed by the solution of sodium chlorid in which the parasites are kept *in vitro* are not hemolytic.

CONCLUSIONS

The failure to demonstrate hemolytic principles in the excretions of the worms when kept *in vitro* appears to favor the view that the hemotoxic substances of ascaris partake of the nature of endotoxins. There is also to be considered the possibility that the death of a worm in the intestine may be followed by a rapid disintegration of its tissues and the liberation of toxic substances before it passes out of the body of the host. Tallqvist (*11*), in fact, has shown in the case of another parasite (*Dibothriocephalus latus*) that the toxic substances are liberated only when the worm disintegrates, which affords a possible explanation why Dibothriocephalus sometimes produces no ill effects on its host, whereas in other instances a severe anemia is present. The fact that in some cases human beings and other animals infested with ascarids remain in apparent good health

while in other cases they show evidences of suffering from such infestation may perhaps be explained in much the same way as the differences observed in cases of infestation with Dibothriocephalus.

The inhibitory effect of the serum on the hemolytic action of the body fluids and extracts of the worms appears to be a direct negation of the view that anemia of animals harboring ascarids is due to the toxic secretions of the worms. It is necessary to remember, however, that a reaction *in vivo* may be very different from a reaction *in vitro*.

Apart from the question of anemia as a result of the absorption of toxins produced by Ascaris, there is the question of anemia as a result of the direct abstraction of blood by the parasite. The opinion that Ascaris is a bloodsucker has been expressed by Schimmelpfennig (9), who based his view largely on the fact that the body fluid of ascaris contains oxyhemoglobin, the source of which presumably is the blood of its host. The view that Ascaris may suck blood is also supported by the structure of the mouth parts of the parasite and the lesions observed in the mucosa of intestines of animals harboring ascaris.

It should be remembered that ascaris is provided with strong chitinous lips, denticulated along their edges. That such buccal armature could succeed in lacerating the smaller blood vessels of the intestine is by no means improbable. Blanchard (2) states that there can be no doubt that *A. lumbricoides* bites the intestinal mucosa. Guiart (7) has shown that *A. conocephala* is often firmly attached to the mucosa of its host; he also states that Leroux found wounds in the intestinal mucosa of man resembling punctures which were apparently produced by *A. lumbricoides*.

Friedberger and Fröhner (6) state that the intestinal mucosa of dogs harboring ascarids often shows evidence of punctures.

The above observations, coupled with the presence of oxyhemoglobin in the worms, a substance which apparently is constantly being excreted by the parasites (to judge from their behavior *in vitro*) and which consequently must be as constantly renewed, appear to favor the view that Ascaris probably supplements its food intake by sucking blood from time to time. The hemolytic substance which is particularly abundant in the intestine of the worms apparently serves the purpose of liberating the oxyhemoglobin from the corpuscles some of which passes into the body fluid of the parasites. In this connection it should be recalled that Ascaris is rich in iron and that this substance enters in considerable quantity into the composition of the eggs (Schimmelpfennig, 9). The significance of the oxyhemoglobin in the body fluid of the worms is not well understood. Whether it merely represents a by-product in the metabolism of the worm and is always excreted as such, or whether it may also first be broken down into simpler compounds with the retention of some of the iron by the tissues of the parasite, still remains to be answered. Whether or not oxyhemoglobin fulfills an important function in the life processes of the worm—perhaps in oxidation—is another question to be solved. In this

connection it is interesting to observe that coincident with the disappearance of oxyhemoglobin from the worms *in vitro* they become sluggish, and that their existence after the complete elimination of this substance is very brief.

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